Multidrug resistance in Gram-negative bacteria that produce extended-spectrum β-lactamases (ESBLs)

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ABSTRACT

In 1983, just two years after the introduction of the oxymino-β-lactams to the market , the first extended-spectrum β-lactamases were isolated in Germany from *Klebsiella pneumoniae* strains. Since then several outbreaks have been reported in many European countries and the USA, and nowadays in several places worldwide the problem seems to reach endemic dimensions, with rates exceeding 50% in some countries, such as Portugal and Turkey. On the other hand not only *K. pneumoniae* but also *Escherichia coli* strains, with *Enterobacter aerogenes* predominating among the other enterobacteriaceal species, are increasingly reported as ESBL producers. In this review types, molecular characteristics, detection methods, epidemiology as well as interventions for therapy and antibiotic strategies to prevent and control infections caused by ESBL-producing microorganisms, are presented and discussed.

Keywords Multidrug resistance, ESBLs, review

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INTRODUCTION

It has been well known for many years that several genera of Gram-negative bacteria possess naturally occurring, chromosomally mediated β-lactamases, which are thought to have evolved from penicillin-binding proteins as a result of the selective pressure exerted by soil organisms producing β-lactams [1]. The first plasmid-mediated β-lactamase isolated from Gram-negative bacteria, which hydrolysed penicillin and penicillin derivatives, such as ampicillin, carbenicillin, piperacillin and first-generation cephalosporins, was TEM-1, described in the early 1960s [2]. This enzyme was found in a blood culture isolate of *Escherichia coli* from a Greek patient named Temoniera, hence the designation TEM. As the latter enzyme was plasmid and transposon mediated, it spread easily to many different species of Gram-negative bacteria [3]. Subsequently, SHV-1 β-lactamase, chromosomally encoded in the majority of *Klebsiella pneumoniae* and plasmid mediated in *E. coli*, was reported [4]. It was just 2 years after the introduction of oximino-β-lactams, such as cefotaxime, ceftazidime and ceftriaxone, and the oximino-monobactam, aztreonam, which were specifically designed to resist the hydrolytic action of β-lactamases, that extended-spectrum β-lactamases (ESBLs) were first isolated in Germany in 1983 from *K. pneumoniae* strains [5]. Since then, several outbreaks have been reported in a number of European countries and the USA, and the problem has reached endemic dimensions in several places worldwide. The selective pressure of the use and overuse of the oximino-cephalosporins, which are widely prescribed for the treatment of serious Gram-negative nosocomial infections, has led to the isolation of a large number of ESBL enzymes worldwide, mainly from different genera of the Enterobacteriaceae [6].

This review focuses on the types, molecular characteristics of ESBLs, the methods for their detection, their epidemiology, and antibiotic strategies to prevent and control their prevalence.

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TYPES AND MOLECULAR CHARACTERISTICS

Most ESBL enzymes contain a serine at the active site and belong to Ambler's molecular class A [7]. A classification scheme devised by Bush *et al*. [8], in 1995, placed β-lactamases into functional groups using their biochemical properties, molecular structure and nucleotide sequence of the genes. According to this scheme, ESBLs are defined as β-lactamases that hydrolyse oximino-cephalosporins and are inhibited by clavulanic acid; they are placed into group 2be. ESBLs are not active against cephamycins, and therefore most strains expressing ESBLs should be susceptible to cefoxitin and cefotetan. However, owing to the loss of an outer membrane porin protein, ESBL-producing strains are often resistant to cephamycins [9,10].

Most ESBLs are TEM and SHV enzyme derivatives characterised by a few point mutations at selected loci within the gene [8,11]. As of 25 January 2005, there were 138 TEM- (TEM-1 to TEM-139) and 62 SHV-type (SHV-1 to SHV-63) β-lactamases, mostly found in *K. pneumoniae* and *E. coli* strains (http://www.lahey.org/studies/webt.htm).

They are being reported with increasing frequency in various genera of Enterobacteriaceae, such as *Salmonella* spp., *Enterobacter aerogenes*, *Proteus mirabilis*, *Providencia rettgeri* and *Morganella morganii*; TEM-42 has been described from *Pseudomonas aeruginosa* and TEM-17 from a blood isolate of *Capnocytophaga ochracea* [12–19]. The first TEM detected enzyme, TEM-1, is still the most commonly encountered β-lactamase in Gram-negative bacteria. TEM-1 is responsible for ampicillin resistance in *E. coli* and ampicillin and penicillin resistance in *Haemophilus influenzae* and *Neisseria gonorrhoea*; it is able to hydrolyse penicillins and first-generation cephalosporins [20]. TEM-2 has a single amino-acid substitution from the original TEM-1 that does not, however, change its substrate profile [21]. The first real ESBL to be described in 1983 was TEM-3 and, in subsequent years, over 100 additional TEMs have been isolated. The combinations of amino-acid substitutions that occur within the TEM enzymes at a limited number of positions result in a variety of subtle alterations, such as the ability to hydrolyse specific oximino-cephalosporins (ceftazidime and cefotaxime), and changes in the isoelectric point (which ranges from 5.2 to 6.5) [8]. A number of amino-acid residues are particularly important for displaying the ESBL phenotype. They include arginine to either serine or histidine at position 164, glycine to serine at position 238 and glutamate to lysine at position 240 [6].

Unlike the TEM-type β-lactamases, there are fewer derivatives of SHV-1. The majority are characterised by the substitution of serine for glycine at position 238, which is critical for the efficient hydrolysis of ceftazidime, whereas a number of variants related to SHV-5 possess a substitution of lysine for glutamate at position 240, leading to efficient hydrolysis of cefotaxime [5,22].

In Greece, 13 years ago, an ESBL (SHV-5 type) responsible for ceftazidime resistance was discovered in *K. pneumoniae* strains [23]. Cross-resistance of the latter strains to aminoglycosides stimulated the search for resistance mechanisms in multiresistant *K. pneumoniae* strains. This investigation was considered to be very important, as empirical treatment of serious nosocomial infections in most Greek tertiary hospitals was usually initiated with a combination of an advanced cephalosporin plus an aminoglycoside. A multiresistant transferable plasmid encoding the SHV-5 β-lactamase, causing unusually high resistance to ceftazidime and aztreonam, and the combination of acetylating enzymes, AAC(6')-I + AAC(3)-I, producing resistance to all clinically available aminoglycosides, was established in *K. pneumoniae* [24].

In the early 1990s, β-lactamases resistant to clavulanic acid and sulbactam inhibition (inhibitor-resistant TEM β-lactamases) were discovered, exhibiting resistance at the clinical level to the β-lactam–β-lactamase inhibitor combinations of amoxicillin–clavulanate, ticarcillin–clavulanate and ampicillin–sulbactam (but inhibition by tazobactam was retained), and subsequently to piperacillin–tazobactam [6,25–27]. Despite the fact that IRTs are not real ESBLs, as are classical TEM and SHV enzyme derivatives, they should be discussed with ESBLs. Therefore, they have been renamed with numerical TEM designations. There are at least 23 distinct IRT enzymes (http://www.lahey.org/studies/webt.htm), primarily detected in France, and found mainly in clinical isolates of *E. coli*, but also in some strains of *K. pneumoniae*, *Klebsiella oxytoca*, *P. mirabilis* and *Enterobacter cloacae* [28,29]. The sites of IRT amino-acid substitutions are distinct from those of ESBL point mutations, occurring at a few specific

amino-acid residues, such as methionine at position 69, arginine at positions 244 and 275 and asparagine at position 276 [30–32]. However, recently, the TEM-50 enzyme, with common amino-acid substitutions to both ESBLs and IRTs, has been described, conferring slight resistance to oximino-cephalosporins [33]. Possibly, this newer enzyme demonstrates a new group of β-lactamases with a more complex phenotype.

The so-called CTX-M enzymes are a relatively new family of plasmid-mediated ESBLs, which preferentially hydrolyse cefotaxime and are better inhibited by tazobactam than by sulbactam and clavulanate (Table 1). These enzymes are not very closely related to TEM and SHV β-lactamases as they show only 40% identity with these enzymes [34]. Such enzymes have been mainly detected in *Salmonella typhimurium* and *E. coli* [6]. It seems that the serine residue at position 237, which is present in CTX-M enzymes, has an important role in their extended spectrum of activity [6]. Strains expressing the CTX-M-type β-lactamases have been isolated worldwide, numbering 29 enzymes, and have been associated with local outbreaks in Japan, South America and eastern Europe; an endemic focus has also been reported from Spain [35–37]. Recently, in Greece, six *E. coli* strains with the ESBL phenotype have been analysed [38]. All were resistant to the combinations of amoxicillin–clavulanate and ampicillin–sulbactam; four were also resistant to cefoxitin, but all were susceptible to piperacillin–tazobactam. In these strains, a new CTX-M enzyme, differing from CTX-M-15 by one amino-acid substitution in position 109 (asparagine to serine), was isolated, conferring a lower level of resistance to ceftazidime compared with cefotaxime (MICs of 16 μg/mL vs. >512 μg/mL).

Fifteen classical OXA-type β-lactamases also belong to the ESBL family. However, they differ from TEM and SHV enzymes by their classification to molecular class D and functional group 2d and their poor inhibition by clavulanic acid [8]. They confer resistance to ampicillin and first-generation cephalosporins, but weak resistance to oximino-cephalosporins, when cloned in Enterobacteriaceae. In order to confer the ESBL phenotype, either of two mutations is required: an asparagine for serine at position 73 or an aspartate for glycine at position 157 [6,39]. In contrast with most ESBL enzymes, they have been mainly isolated in *P. aeruginosa* and provide high-level resistance to ceftazidime [40]. It should be pointed out that many new members of the OXA β-lactamases, such as OXA-20, OXA-22, OXA-24, OXA-25, OXA-26, OXA-27 and OXA-30, do not belong to the ESBL family [41–45].

Almost 10 years ago, PER-1, the first of a series of novel ESBLs not closely related to any of the well-known families of β-lactamases, was reported [46]. It was discovered in Turkey from *P. aeruginosa* strains and later from strains of *S. typhimurium* and *Acinetobacter baumannii* [47–49]. A related enzyme, PER-2, with 86% amino-acid homology with PER-1, was exclusively reported from Argentina in 1996 [50]. Subsequently, at least seven novel unrelated ESBLs have been reported from several parts of the world, isolated from different species of Enterobacteriaceae (Table 2).

METHODS FOR DETECTING ESBLs

The steadily increasing prevalence of ESBL-producing Enterobacteriaceae worldwide has created a great need for accurate techniques of laboratory testing for identification. It is very important to emphasise that, according to the NCCLS interpretive definitions, ESBLs do not always increase MICs to levels characterised as resistant [51,52]. On the other hand, according to the NCCLS, an inhibitory zone of ≤22 mm for ceftazidime, ≤27 mm for cefotaxime and aztreonam and ≤25 mm for ceftriaxone should raise suspicion of ESBL production [52]. The lack of sensitivity and specificity of traditional dilution or disc diffusion susceptibility tests for the detection of ESBLs has prompted the development of a variety of fairly accurate methods based on the observation that, in clinical microbiology tests, ceftazidime or cefotaxime in combination with a β-lactamase inhibitor, usually clavulanate, which inhibits ESBLs, reduces the level of resistance to the former cephalosporins. The following tests based on clinical microbiological techniques have been applied.

1 The double-disc approximation test [53]. This was one of the first detection methods and is based on the Kirby–Bauer diffusion technique. A susceptibility disc containing amoxicillin–clavulanate is placed in the centre of a Mueller–Hinton agar plate, and a disc containing one of the oximino-β-lactam antibiotics (e.g., ceftazidime) is placed 30 mm (centre to centre) from the amoxicillin–clavulanate disc. The clavulanate diffuses through the agar and inhibits the

Table 1. Published CTX-M-type extended-spectrum β-lactamases (http://www.lahey.org/studies/webt.htm, last update 25 January 2005)

Cluster	β-lactamase	pI	Amino acid change	Origin	Host
			Compared with CTX-M-1 [116]		
CTX-M-1	CTX-M-1	8.9	–	Germany, Italy	*Escherichia coli*
	CTX-M-3	8.4	Val77Ala, Asp114Asn, Ser140Ala Asn288Asp	Poland	*Citrobacter freundii*, *Escherichia coli*
	CTX-M-10	8.1	Ala27Val, Arg38Gln, Val77Ala, Asp114Asn, Ser140Ala, Asn288Asp	Spain	*Escherichia coli*
	CTX-M-12	9.0	Thr12Ala, Val77Ala, Asn89Ser, Asp114Asn, Ser140Ala, Val278Ile, Asn288Asp	Kenya	*Klebsiella pneumoniae*
	CTX-M-15	8.6	Asp240Gly	India	*Escherichia coli*
	CTX-M-23	8.9	Asp114Asn, Ser140Ala, Pro167Thr	Germany	*Escherichia coli*, *Klebsiella pneumoniae*
	CTX-M-32	9.0	Asp240Gly	Spain	*Escherichia coli*, *Klebsiella pneumoniae*
			Compared with CTX-M-2 [116]		
CTX-M-2	CTX-M-2	7.9	–	Argentina	*Salmonella enterica*
	CTX-M-4	8.4	Leu48Gln, Arg61Val, Lys98Arg, Lys99Ala, Ala125Gly, Thr171Ser, Leu225Met, Val230Gly	Russia	*Salmonella typhimurium*
	CTX-M-5	8.8	Ala27Thr, Val230Gly, Glu254Ala, Ile278Val	Latvia	*Salmonella typhimurium*
	CTX-M-6	8.4	Arg61Leu, Lys99Ala, Ala125Gly, Thr171Ser, Ser228Cys, Ile276Val	Greece	*Salmonella typhimurium*
	CTX-M-7	8.4	Arg61Val, Lys98Arg, Lys99Ala, Glu121Gln, Ala125Gly, Thr171Ser, Val230Gly, Ile276Val	Greece	*Salmonella enterica*
	CTX-M-20	8.3	Ile279Phe	France	*Proteus mirabilis*
	CTX-M-31	8.2	Thr162Ser	Argentina	*Providencia sp*, *E.coli*
	Toho-1	7.8	Ser39Arg, Ser274Arg	Japan	*Escherichia coli*
CTX-M-8	CTX-M-8	7.6	AAC, 44, p1936-42 [117]	Brazil	*Proteus mirabilis*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter amalonaticus*
	CTX-M-25		AAC, 48, p 4829-4834 [120]		
	CTX-M-26		AAC, 48, p 4829-4834 [120]		
			Compared wtih CTX-M-9 [118]		
CTX-M-9	CTX-M-9	8.0	–	Spain	*Escherichia coli*
	CTX-M-13	8.2	Val3Met, Val53Lys, Ala154Glu, Ala231Val		*Enterobacter cloacae*
	CTX-M-14	8.1	Ala231Val	Spain	*Escherichia coli*
	CTX-M-16	8.2	Asp240Gly	Brazil	*Escherichia coli*
	CTX-M-17		Ala231Val, Glu289Lys	Vietnam	*Klebsiella pneumoniae*
	CTX-M-18	8.0	Ala231Val	France	*Escherichia coli*, *Klebsiella pneumoniae*
	CTX-M-19	8.0	Pro167Ser, Ala231Val	France	*Klebsiella pneumoniae*
	CTX-M-27	8.2	Ala231Val, Asp240Gly	France	*Escherichia coli*
	Toho-2	7.7	Phe8Ser, Ala21Pro, Pro22Thr, Pro183Ala, Ala187Gly, Ala231Val	Japan	*Escherichia coli*
CTX-M-21	CTX-M-21	8.4	FEMS Microb Lett. 209, 161-168, 2002[119]	France	*Escherichia coli*

Table 2. Novel unrelated extended-spectrum β-lactamases (http://www.lahey.org/studies/webt.htm)

β-Lactamase	Related to	pI	Substrate	Origin	Host
BES-1	Penicllinase from *Yersinia enterocolitica*	7.5	CTX, CAZ, ATM	Brazil	*Serratia marcescens*
FEC-1		8.2	CTX	Japan	*Escherichia coli*
GES-1	Penicillinase from *Proteus mirabilis*	5.8	CAZ	French Guiana	*Klebsiella pneumoniae*
CME-1	VEB-1	>9.0	CAZ		*Chryseobacterium meningosepticum*
PER-1	PER-2	5.4	CAZ	France	*Pseudomonas aeruginosa*
PER-2	PER-1	5.4	CAZ	Argentina	*Salmonella typhimurium*
SFO-1	AmpA from *Serratia fonticola*	7.3	CTX	Japan	*Enterobacter cloacae*
TLA-1	CME-1	9.0	CAZ, CTX, ATM	Mexico	*Escherichia coli*
VEB-1	PER-1, PER-2	5.35	CAZ, ATM	Vietnam/Thailand	*Escherichia coli*
IBC-1	IBC-2, GES-1	6.9	CAZ	Greece	*Enterobacter cloacae*
IBC-2	IBC-1, GES-1	5.8	CAZ	Greece	*Pseudomonas aeruginosa*

CTX, Cefotaximase; CAZ, Ceftazidime; ATM, Aztreonam.

β-lactamase surrounding the ceftazidime disc. Enhancement of the zone of the ceftazidime disc on the side facing the amoxicillin–clavulanate disc is interpreted as a positive test.

2 The Etest ESBL strip (AB Biodisk, Solna, Sweden) [6]. In this method the zone of inhibition is read from two halves of the strip containing ceftazidime alone or ceftazidime plus clavulanate. A decrease in the MIC of ceftazidime of more than three dilutions in the presence of clavulanate is interpreted as a positive test. However, the Etest ESBL strip is sometimes difficult to interpret with weak enzyme producers that are expressed with low ceftazidime MICs, because clavulanate may diffuse to the side that contains ceftazidime alone.

3 Commercialised tests with discs containing clavulanate plus ceftazidime or cefotaxime (10 μg plus 30 μg, respectively). These methods have been shown to detect accurately the presence of ESBLs when an increase of ≥5 mm in the inhibitory zone of ceftazidime or cefotaxime plus clavulanate compared with controls (i.e., single cefotaxime or ceftazidime disc) is observed [54,55].

4 The three-dimensional test. This method is considered to be very sensitive for the detection of ESBLs, but is technically difficult and labour intensive, requiring experienced clinical microbiologists to interpret the results [56].

5 The automated ESBL microbial susceptibility test system Vitek (BioMérieux, Marcy l'Etoile, France) [6]. This method utilises either ceftazidime or cefotaxime alone and in combination with clavulanic acid (4 μg/mL). A predetermined reduction in growth in wells containing clavulanate, compared with those containing each single drug, indicates the presence of an ESBL. Sanders *et al*. [57], in a study of *Klebsiella* spp. and *E. coli* possessing well-known β-lactamases, showed that the Vitek ESBL test was 99% sensitive and specific for the detection of ESBLs. However, Tzelepi *et al*. [58] reported that the Vitek ESBL detection test failed to detect the majority of ESBL-producing strains of *Enterobacter* spp. Moreover, in a European survey, after applying the Vitek test, 37% of ESBL-producing organisms were mistakenly reported as being susceptible to extended-spectrum cephalosporins (ESCs) [59].

6 Recently, the NCCLS has recommended initial screening by testing for growth in a broth medium containing 1 μg/mL of one of five extended-spectrum β-lactam antibiotics [52]. A positive result should raise suspicion of the presence of an ESBL. This initial screening should be followed by a phenotypic confirmatory test for the determination of MICs of either ceftazidime or cefotaxime, with and without the presence of clavulanic acid (4 μg/mL). A decrease in the MIC of three or more twofold dilutions in the presence of clavulanate is indicative of the presence of an ESBL.

It should be pointed out that, if an ESBL is detected, the strain should be reported as non-susceptible to all ESCs and aztreonam, regardless of the susceptibility test result [52]. However, it should be noted that false positive ESBL results can occur when strains produce high SHV-1 levels or when an AmpC-type enzyme is expressed in the same isolate [60–63].

To conclude, it seems that the double-disc approximation test and the broth dilution MIC reduction method are the easiest and most

cost-effective for application by clinical laboratories.

In order to identify the specific ESBL expressed in a clinical isolate, more laborious and complicated methods are required. The determination of the isoelectric point was used in the past, but is no longer adequate to characterise the exact ESBL, as different β-lactamases possess identical isoelectric points. The following molecular detection methods have been applied [6]: (i) specific DNA probes; (ii) polymerase chain reaction (PCR) with oligonucleotide primers; (iii) oligotyping; (iv) PCR followed by restriction fragment length polymorphism analysis; (v) PCR–single-strand conformational polymorphism analysis; (vi) ligase chain reaction; (vii) nucleotide sequencing. All of these methods share several advantages and drawbacks, except the latter, which represents the standard, detecting all specific β-lactamase genes present in a strain [64]. However, it is labour intensive and technically challenging, whereas manual methodology is difficult to interpret.

According to the NCCLS guidelines, and as already mentioned, many ESBL-producing isolates are not always phenotypically resistant to oximino-cephalosporins. However, patients suffering from infections caused by ESBL-producing organisms are at risk of treatment failure if an ESC is prescribed [65,66]. Therefore, it is imperative for the clinical microbiology laboratory to identify isolates that possess increased MICs (≥ 2 μg/mL) to oximino-cephalosporins, even though they may be equal to or below the susceptibility breakpoint (MIC ≤ 8 μg/mL), and subsequently to implement one of the reported methods to detect ESBLs [51,67,68]. On the other hand, in order to avoid misleading results for the clinician, and according to NCCLS recommendations, it is very important that any *Klebsiella* sp. or *E. coli* that is confirmed as an ESBL producer should be reported as being resistant to all cephalosporins, penicillins and aztreonam, regardless of the susceptibility test result [52].

The inoculum effect demonstrated by many ESBL-producing strains should also be of great concern. Medeiros and Crellin [69] found that the MIC rose dramatically when the inoculum in the susceptibility tests was increased from 10^5 to 10^7 cfu/mL, an effect also demonstrated in animal models of intra-abdominal abscesses and infectious endocarditis. In addition, in many human infections, such as meningitis, endocarditis, septic arthritis, osteomyelitis and various abscesses, the bacterial load may even exceed such high inoculum levels, reaching colony counts of 10^9–10^{10} cfu/mL or gram of tissue [70,71]. Recently, Thomson and Smith-Moland [72] studied *in vitro* the influence of the inoculum effect on meropenem, cefotetan, cefotaxime, ceftazidime, ceftriaxone, cefepime, aztreonam and piperacillin against strains of *Klebsiella*, *Escherichia*, *Enterobacter*, *Citrobacter*, *Serratia*, *Morganella* and *Proteus* spp. that produced 22 different ESBLs. An inoculum effect, defined as an eight-fold or greater MIC increase on testing with the higher inoculum (10^5 cfu/mL vs. 10^7 cfu/mL), was most consistently detected with cefepime, cefotaxime and ceftriaxone, and less frequently with meropenem and cefotetan, whereas piperacillin–tazobactam was intermediate between these two groups of agents. Despite previous suggestions, the latter in-vitro results confirm that, until prospective reliable clinical results exist, cefepime should not be considered as the therapy of choice in serious infections in which ESBL strains are implicated. With regard to piperacillin–tazobactam, inoculum effects occurred in tests with strains producing SHV-derived ESBLs, and only occasionally in tests with strains producing TEM-derived ESBLs [72]. The relevance of these in-vitro findings must be tested with reliable clinical data.

EPIDEMIOLOGY

The ESBL phenomenon was first reported in Germany almost 20 years ago, but it did not take long to become established elsewhere, including in the USA and Asia [5,6]. In the USA, *K. pneumoniae* resistance rates to ceftazidime increased from 4% in 1990 to 14% in 1993 [73]. However, in a study by Fridkin *et al*. [74], involving intensive care unit (ICU) patients in the USA in 1996–97, only 3.7% of *K. pneumoniae* were reported to be resistant to third-generation cephalosporins, whereas in the NNIS/ICARE and SENTRY studies (1999 and 1997–99), respectively, the corresponding values were 9% and 10%, respectively [74,75]. A surveillance of antibiotic resistance in European ICUs for the years 1990–1999 revealed a wide variation in ESBL producers, with the highest prevalence of 73% observed in Turkey. ICUs in Portugal, France and Russia also showed a significant problem with resistance rates of 34%,

36% and 33%, respectively [76]. In contrast, Belgian, German, Spanish and Swedish ICUs had only a 1–4% prevalence of suspected ESBL-producing *Klebsiella* spp. for the years 1994–95. In a study organised by Livermore and Yuan [59] in 1994, a high prevalence of ESBL in *Klebsiella* spp. was observed in Belgium (31%), France (24%), Italy (17%), the Netherlands (16%), Portugal (49%) and Turkey (59%), with no ESBL detected in the UK, and low rates in Spain (1%), Greece (5%) and Germany (9%). However, the data from the most recent European SENTRY study, performed mainly in southern Europe, showed a 39% mean prevalence of suspected ESBL-producing *Klebsiella* spp., with the worst situation reported, once more, from Turkey [76]. The large variation amongst the participating countries in Europe is probably indicative of ESBL outbreaks.

With regard to ESBL-producing *E. coli* strains, Turkey is the country with the highest resistance rate (26%), while, in the European SENTRY study, 9% of *E. coli* strains, mostly collected from southern European countries, were resistant to ceftazidime [76]. In the study by Hanberger *et al*. [76], 0–4% of *E. coli* isolates reported in European studies were resistant to ceftazidime, a rate similar to the 3% prevalence of ceftazidime resistance reported from ICUs in the USA. In Japan, in a recent survey of 196 institutions across the country, only $<0.1\%$ of *E. coli* and 0.3% of *K. pneumoniae* strains expressed an ESBL, whereas corresponding figures for ESBL production in *E. coli* and *K. pneumoniae* from Korea, Taiwan and Hong Kong were 4.8%, 8.5% and 12%, respectively [77–80].

Surveys in both the USA and Europe have indicated that SHV-4 and SHV-5 are the most common ESBLs, while the predominant TEM types in the USA (TEM-10, TEM-12, TEM-26) are different from those in Europe (TEM-3, TEM-24) [81,82]. However, recently, TEM-10 was reported with the same frequency from Portugal and the UK [6,83,84]. Outbreaks of TEM-47 in Poland and TEM-52 in Korea have also been reported recently, while SHV-5 β-lactamase is encountered worldwide, with reports from France, Croatia, Greece, Hungary, Poland, the UK, the USA and South Africa [6]. It therefore seems that ESBLs arise and disseminate locally, but the spread of particular ESBL types or ESBL-producing strains can also occur between hospitals that exchange patients. Recently, in several Greek ICUs, a multiresistant *K. pneumoniae* strain with borderline susceptibility to gentamicin (MIC = 4 μg/mL) and sensitive only to colimycin caused local outbreaks; it has already become endemic in some ICUs. Such strains harbour, on the same plasmid, not only SHV-5, but also a VIM-1 like enzyme conferring additional resistance to carbapenems (H. Giamarellou, unpublished observations, 2004).

With regard to non-*E. coli*, non-*Klebsiella* Enterobacteriaceae species producing ESBLs, *E. aerogenes* seems to predominate. Nosocomial outbreaks in ICUs caused by cross-colonisation have been reported in France, Belgium and Austria, where the dissemination of epidemic clones of multiresistant *E. aerogenes* harbouring ESBLs, and particularly TEM-24, has been observed [6,76,85].

Recently, outbreaks of organisms producing multiple β-lactamases have been described. This combination comprises non-ESBL class A enzymes and chromosomal AmpC-type enzymes together with ESBLs. Similar strains express resistance to β-lactam–β-lactamase inhibitor combinations, cephamycins, oximino-cephalosporins, aztreonam and even carbapenems [6]. Surprisingly, a high association with resistance to quinolones has been observed in strains producing ESBL enzymes. Paterson *et al*. [86] reported that, among ciprofloxacin-resistant strains, 60% were ESBL producers, compared with 16% in ciprofloxacin-susceptible strains ($p = 0.0001$).

The epidemiological characteristics of ESBL outbreaks have been studied by several investigators. The risk factors associated with the acquisition of ESBL-producing organisms are described in Table 3 [87–93]. Lautenbach *et al*. [94] studied

Table 3. Risk factors associated with extended-spectrum β-lactamase-producing organisms in case–control studies [87–93]

Nosocomial infection
Previous administration of any antibiotic
Previous administration of ceftazidime or aztreonam
High Acute Physiology and Chronic Health Evaluation (APACHE) II score
Ventilatory assistance
Urinary catheter
Arterial catheter
Central venous catheter
Gut colonisation
Emergency abdominal surgery
Gastrostomy or jejunostomy tube
Length of hospital stay
Length of intensive care unit stay
Age
Nursing home residence
Low birth weight

the risk factors for infection and the impact of resistance on outcomes when ESBL-producing *E. coli* and *K. pneumoniae* were implicated. Thirty-three patients with infection due to ESBL-producing *E. coli* or *K. pneumoniae* (case patients) were compared with 66 matched controls. Total previous antibiotic use was the only independent risk factor for ESBL-producing *E. coli* or *K. pneumoniae* infection (odds ratio, 1.10; 95% confidence interval, 1.03–1.18; $p = 0.006$). Case patients were treated with an effective antibiotic a median of 72 h after infection was suspected, compared with a median of 11.5 h for controls ($p < 0.001$). Infections caused by ESBL-producing *E. coli* or *K. pneumoniae* were associated with a significantly longer duration of hospital stay and greater hospital charges ($p = 0.01$ and $p < 0.001$, respectively). The authors emphasised that ESBL-producing *E. coli* and *K. pneumoniae* infections have a significant impact on several important clinical outcomes, and efforts to control outbreaks of infection with similar strains should emphasise the judicious use of all antibiotics as well as barrier precautions to reduce their spread.

INTERVENTIONS FOR THE THERAPY, CONTROL AND PREVENTION OF INFECTIONS CAUSED BY ESBL-PRODUCING GRAM-NEGATIVE MICROORGANISMS

ESBL-producing Enterobacteriaceae are, as a rule, resistant to all cephalosporins and extended-spectrum penicillins, including the monobactam aztreonam, while resistance to trimethoprim–sulfamethoxazole and aminoglycosides is frequently co-transferred on the same plasmid [95]. Therefore, therapeutic choices are limited. However, several reports in the literature describe the results obtained after the prescription of cefotaxime or ceftazidime for ESBL-producing microorganisms. One of the larger studies was reported by Meyer *et al*. [96], who documented a 2-year outbreak of ESBL-producing *K. pneumoniae* with 155 colonised or infected patients. Therapy and outcome were evaluated in 43 infected patients. Thirteen of 13 patients with invasive infection, who did not receive therapy directed against ESBLs, died, whereas two patients with non-bacteraemic bacteriuria responded to cephalosporins. Naumovski *et al*. [97] reported their experience with ESBL-producing *K. pneumoniae* bacteraemia in four cancer patients. Two patients who received ceftazidime alone died, but two patients in whom tobramycin was added to ceftazidime survived. In a global study of more than 200 cases of *K. pneumoniae* bacteraemia from seven hospitals on five continents, 15% of cases with ESBL isolates were compared with 85% of cases with non-ESBL isolates [91]. In 84% of ESBL cases, bacteraemia was hospital acquired, compared with only 43% of non-ESBL cases. In 31% of ESBL cases, a third-generation cephalosporin had recently been prescribed, compared with only 3% of non-ESBL cases. However, piperacillin–tazobacam and imipenem use were not associated with ESBL infections. The crude mortality rate of patients with ESBL bacteraemia was 46%, compared with 34% in the non-ESBL group. Finally, it is noteworthy that, amongst patients with ESBL-producing *K. pneumoniae* bacteraemia, who received initial empirical therapy to which the organism was resistant, 75% died, compared with 28% who received initial empirical therapy to which the organism was susceptible.

In an attempt to clarify whether screening of *Klebsiella* spp. or *E. coli* for ESBL was clinically necessary, Paterson *et al*. [98] performed a prospective, multinational study of *K. pneumoniae* bacteraemia, and identified 10 patients with ESBL-producing strains who were treated with cephalosporins and whose infecting organisms were not resistant *in vitro* to the utilised cephalosporin. In the same study, 26 similar cases of severe infections which had previously been reported were also reviewed. In a total of 32 evaluated cases, 100% (4/4) patients experienced clinical failure when the MICs of the cephalosporin used for treatment were in the intermediate range, and 54% (15/28) experienced failure when the MICs of the cephalosporin used for treatment were in the susceptible range. The authors concluded that it is clinically important to detect ESBL production by *Klebsiella* spp. or *E. coli*, even when cephalosporin MICs are in the susceptible range (≤ 8 μg/mL), and to report ESBL-producing organisms as resistant to aztreonam and all cephalosporins.

Wong-Beringer *et al*. [99] recently investigated the molecular correlation of treatment outcomes in bloodstream infections caused by *E. coli* and *K. pneumoniae* with reduced susceptibility to

ceftazidime. They described the largest treatment experience of a non-outbreak series of bloodstream infections caused by strains of *E. coli* (23 episodes) and *K. pneumoniae* (13 episodes) with ceftazidime MICs of ≥2 μg/mL. *E. coli* isolates produced a greater variety of β-lactamase types than did *K. pneumoniae* isolates, among which ESBL production was predominant. Five ESBL types were identified: TEM-12, TEM-71, TEM-6, SHV-12 and SHV-5. Most patients were treated empirically with an ESC-based regimen. A favourable response to treatment with a non-ceftazidime ESC was observed when the causative pathogen produced either TEM-6 or TEM-12, whereas ceftazidime treatment was associated with failure of therapy in all patients. Despite the limited clinical success, ESCs were not recommended for the treatment of serious infections caused by ESBL-producing strains. With regard, in particular, to the choice of cefepime as empirical therapy for Gram-negative bacillary infections in centres in which ESBL-producing bacteria predominate, the fact that the incidence of Enterobacteriaceae also carrying plasmid-mediated AmpC-type β-lactamases is increasing mandates the need for prospective studies to explore its specific role in such situations [100]. Until then, and despite favourable in-vitro susceptibility results, cefepime should not be recommended.

Initially, it was believed that TEM and SHV ESBLs did not hydrolyse the cephamycins; therefore, cefoxitin and cefotetan could be administered for the therapy of proven or suspected infections with ESBL producers. However, it seems that, quite often, such strains also possess a plasmid-mediated AmpC-type β-lactamase that effectively hydrolyses cephamycins. In addition, porin-deficient mutants of *K. pneumoniae* have been described which are selected *in vitro* during therapy with cefoxitin or cefotetan [10,68].

With regard to the critical question of 'whether β-lactamase inhibitors are of therapeutic value in the case of infections caused by ESBL-producing *K. pneumoniae*', there has been much consideration. Piroth *et al*. [101] conducted a case–control study in an ICU in order to investigate the risk of acquisition of ESBL strains, with special reference to therapy with β-lactamase inhibitors and resuscitation procedures. Fifty-one patients colonised or infected with ESBL-producing *K. pneumoniae* (cases) were matched with 51 non-colonised patients (controls). The duration of intubation was significantly longer for cases than for controls, while the duration of β-lactamase inhibitor therapy was significantly shorter. By means of multivariate analysis, intubation was the only risk factor identified, while β-lactamase inhibitor therapy, 80% of which included clavulanic acid and 20% tazobactam, was shown to be a protective factor. The authors concluded that, in ICUs, the preferential use of β-lactamase inhibitors may help control the emergence and spread of these pathogens if essential hand-washing and isolation procedures are adhered to. It has also been reported that the administration of β-lactamase inhibitors may facilitate, at least *in vitro*, their reverse mutation into less harmful enzymes. However, clinical isolates of ESBL-producing *K. pneumoniae*, because of the hyperproduction of ESBL, have been proven to be less susceptible to β-lactam–β-lactamase inhibitor combinations, and the selection of Gram-negative bacteria producing AmpC-type β-lactamases is always a risk [102].

When ESBL isolates are susceptible *in vitro*, therapy with a fluoroquinolone should be satisfactory *in vivo* [95]. However, as already mentioned, fluoroquinolone resistance often coexists in ESBL-producing organisms, probably due to pre-existing risk factors, such as exposure to antimicrobial agents that are given to hospitalised patients who acquire such strains [102,103]. A recent report is of special concern for clinicians, as it has documented a transferable fluoroquinolone resistance in *K. pneumoniae* on a plasmid that also encodes an AmpC-type β-lactamase.

The literature refers to several scattered cases of patients successfully treated with a carbapenem, who were mostly suffering from bacteraemia or meningitis caused by ESBL-producing microorganisms [100]. On the other hand, for similar strains, in-vitro susceptibility results reported imipenem MICs of ≤1.0 μg/mL [100]. It is still uncertain whether combination therapy is necessary and which antibiotic, when combined with a carbapenem, will achieve an optimal outcome *in vivo*. In-vitro combinations of imipenem with amikacin or ciprofloxacin against blood isolates of ESBL-producing *K. pneumoniae* did not result in synergistic interactions [100]. Because of insufficient clinical information, many investigators prefer imipenem and meropenem as the drugs

of choice for serious infections due to ESBL-producing strains. However, Wong-Beringer *et al*. [99] suggested that, in the case of a non-outbreak situation, clinicians, in order to preserve the therapeutic value of carbapenems, need not administer a carbapenem. Based on institutional patterns of susceptibility results, piperacillin–tazobactam, a fluoroquinolone or an aminoglycoside would be preferable. In contrast, in the case of life-threatening infections or in an outbreak setting, empirical therapy would call for a carbapenem. However, it is noteworthy that, in centres with ESBL outbreaks, the frequency of isolating carbapenem-resistant *P. aeruginosa* and *Acinetobacter* spp. increased dramatically as a result of the increased use of this class of antibiotic [103]. For similar strains, only colimycin remains active *in vitro* with moderate in-vivo results [104,105]. Clinicians should also be aware that, during therapy with a carbapenem, bacteria intrinsically resistant to imipenem, such as *Stenotrophomonas maltophilia* and vancomycin-resistant *Enterococcus faecium*, can be selected.

It is evident that no prospective studies exist to elucidate the various treatment options and outcomes for serious infections caused by ESBL-producing organisms. Therefore, appropriately organised prospective trials in centres with endemic ESBL-producing bacteria are urgently required.

The epidemiological characteristics of ESBL outbreaks indicate that the problem may be attributed to clonal dissemination, antibiotic selective pressure on plasmid dissemination amongst distinct clones, or both. One of the earliest ESBL outbreaks was reported in Massachusetts by Rice *et al*. [95], concerning a chronic case facility. ESBLs were detected in five different species, i.e., *K. pneumoniae*, *E. coli*, *Enterobacter agglomerrans*, *Citrobacter divs*. and *Serratia* spp., indicating resistant plasmid dissemination. In another study involving 43 USA medical centres in 26 states, extensive strain diversity was documented, whereas, in France, five nosocomial outbreaks in three hospital wards involved four distinct strains, TEM-3 ESBL being responsible for plasmid dissemination and SHV-4 for clonal dissemination [106]. Therefore, both isolation procedures (e.g., gloves, gowns) and application of hand hygiene, according to the recent CDC guidelines, as well as appropriate antibiotic utilisation policies, should be considered [107]. Reasons to control ESBL producers in particular include: (i) the potential transfer of multiple antibiotic resistance as a result of genes found on large plasmids that may also carry resistance determinants to other classes of antibiotics, such as aminoglycosides and sulphonamides; (ii) their association with nosocomial outbreaks characterised by high morbidity and mortality rates; (iii) the need to limit the excessive use of broad-spectrum antibiotics; and (iv) the need to limit outbreaks by controlling resistance. In addition, as we face the so-called 'End of Antibiotics' era, the application of various methods to control antimicrobial use, in an effort to reverse resistance, is of extreme importance [108]. Selective removal, control or restriction and, recently, cycling of antibiotics, have been suggested [109].

The value of restriction policies to reverse resistance caused by ESBLs has been proven in several studies. Rice *et al*. [110], in 1996, described the control of a ceftazidime-resistant *K. pneumoniae* outbreak at the Cleveland VA Medical Center which reached almost 30% in 1994. Interventions included: (i) encouragement of avoidance of ceftazidime; (ii) education of hospital staff concerning the outbreak; and (iii) selection of piperacillin–tazobactam as an alternative to ceftazidime. The latter choice was based on the antimicrobial activity of piperacillin–tazobactam, which is at least as broad as the ceftazidime antimicrobial spectrum, and, in contrast with a cephalosporin agent, covers also enterococci and resistant *Enterobacter* spp., simultaneously avoiding imipenem. After replacing 50% of ceftazidime use with use of piperacillin–tazobactam, resistance rates decreased from 28% to 10%. In this study, no emphasis or increase in compliance with infection control measures was observed, suggesting that antibiotic restriction was the most important intervention in the clonal outbreak. In an effort to determine whether restriction of the cephalosporin class would reduce the incidence of infection or colonisation by cephalosporin-resistant *Klebsiella* spp., and to determine whether the restriction of cephalosporins would further increase imipenem use and resistance in *P. aeruginosa*, Rahal *et al*. [111] conducted a before and after comparative 2-year trial at a New York hospital. A new antibiotic guideline excluded the use of cephalosporins, except for paediatric infection, single-dose surgical prophylaxis, acute bacterial meningitis, spontaneous bacterial peritonitis and outpatient gonococcal infection. All other cepha-

losporin use required prior approval by the infectious disease section. During the study, a significant decrease in overall cephalosporin use was noted (80.1%), with a 72.5% decrease in ceftazidime consumption; this was followed by a significant decrease in ceftazidime-resistant *Klebsiella* isolates (44%), including both infecting strains and colonisers [combined ICUs, 70.9% ($p < 0.001$); surgical ICU, 87.5% ($p < 0.001$)]. However, imipenem use was increased by 140.6%, followed by a subsequent increase of 68.7% in imipenem-resistant *P. aeruginosa* ($p < 0.01$).

Pena *et al*. [92] reported their results after an outbreak of ESBL-producing *K. pneumoniae* in Barcelona, during which 145 patients were colonised or infected (72% in ICU) and 63% developed infection, with primary bacteraemia in 43%. Interventions during more than a 2-year period included: (i) the use of aprons and cohorting, with heightened awareness of hand-washing and glove use; (ii) education initiatives; (iii) restriction of the use of oximino-cephalosporins; and (iv) the empirical use of imipenem or piperacillin–tazobactam for nosocomial ICU infections. After reduction of oximino-cephalosporins by 87%, ESBL-carrying strains, predominating in 40% of isolates, almost disappeared.

Patterson *et al*. [112], in Texas, studied the association of antibiotic utilisation measures and the control of multidrug-resistant (MDR) *K. pneumoniae* after emergence in two hospitals in their medical centre. Clonal strain dissemination was the major mechanism of emergence at hospital A; emergence was polyclonal at hospital B. Antibiotic utilisation interventions at both institutions included physician education regarding the association of ceftazidime use and MDR *K. pneumoniae*. At hospital A, ceftazidime use decreased from 4.301 g in the pre-intervention period to 1.248 g in the post-intervention period, with an increase in piperacillin–tazobactam from 12.455 g to 17.464 g. Ceftazidime resistance in *K. pneumoniae* decreased from 22% to 15% ($p < 0.05$), whereas piperacillin–tazobactam resistance decreased from 36% to 19% ($p < 0.05$). At hospital B, ceftazidime use decreased from 6.533 g in the pre-intervention period to 4.792 g in the post-intervention period, with a piperacillin–tazobactam increase from 58.691 g to 67.027 g. Resistance in *K. pneumoniae* decreased from 10% to 5% ($p < 0.05$) and from 22% to 14% ($p < 0.05$) for ceftazidime and piperacillin–tazobactam, respectively. Follow-up data showed a continued decrease in piperacillin–tazabactam resistance despite increased use at both hospitals. The authors concluded that antibiotic restriction measures may be particularly important for the control of MDR *K. pneumoniae*, whether emergence is clonal or polyclonal, basing their explanation for the decrease in piperacillin–tazobactam resistance, despite the increase in consumption, on the decrease in the selection and cross-transmission of ESBL-producing *K. pneumoniae* isolates at hospital B and the decrease in selection and cross-transmission of AmpC-type β-lactamase-producing isolates at hospital A.

Recently, in a general ICU in Italy, Luzzaro *et al*. [113] described a nosocomial outbreak of MDR *P. aeruginosa* producing the novel PER-1 ESBL. Although the outbreak was controlled after the application of strict hygiene measures, ulcer disinfection with mercurochrome or silver nitrate and broad application of carbapenems, carbapenem-resistant strains of *Stenotrophomonas maltophilia* and *Pseudomonas putida* producing VIM-1 emerged in the unit.

In Greece, after the application of a 'restricted antibiotic programme' in a tertiary hospital in Athens that included all advanced antibiotics, i.e., third- and fourth-generation cephalosporins, carbapenems, monobactams, fluoroquinolones and glycopeptides, total consumption decreased by 44% (16.2 vs. 10.7 daily defined doses), with a subsequent decrease in ceftazidime- and piperacillin–tazobactam-resistant *K. pneumoniae* strains producing ESBLs from 31% to 15% ($p < 0.001$) and from 34% to 20% ($p < 0.001$), respectively [114]. Cycling of antimicrobial agents in the reported studies was not adequate to determine whether any meaningful impact on resistance occurs as a result of a cycling programme [109]. However, Raymond *et al*. [115] concluded that the implementation of a quarterly empirical antibiotic rotation schedule in an ICU was feasible, and was associated with significant reductions in the incidence of infection, antibiotic-resistant organism infection and infectious mortality without an increase in antibiotic cost.

CONCLUSION

There is no doubt that ESBL-producing Enterobacteriaceae, because of their multiresistant

pattern, create a therapeutic dilemma for the clinician. Based on cumulative clinical experience, carbapenems and piperacillin–tazobactam seem to be the antibiotics of choice for the treatment of serious infections. However, carefully controlled prospective studies to determine the preferable antibiotic are lacking. For the time being, infection control measures and, particularly, compliance with hand hygiene guidelines, as well as appropriate antibiotic policies, including the control of the widespread use of advanced cephalosporins, are urgently required to prevent and to ameliorate the ever-increasing problem of the emergence of MDR ESBL-producing Gram-negative microorganisms.

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